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# BACILLUS MURIS AS THE ETIOLOGICAL AGENT OF PNEUMONITIS IN WHITE RATS AND ITS PATHOGENICITY FOR LABORATORY ANIMALS.*

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*B. muris* was first described by Klein[1] in 1900 as the chief organism found in two cases of pneumonia of white rats. The original report is as follows:

> Klein described a new pathogenic microbe belonging to the group of diphtheroid bacilli. It was found in white rats, causing in these animals extensive consolidation of the lungs. In the hepatized parts, the alveoli and the infundibula and bronchi were filled with and distended by fibrinous exudation, red and white corpuscles, and continuous masses of the above microbe. Microscopic specimens and lantern slides of the microbe, as it occurred in the lungs and under cultivation, were shown. The morphological, including Neisser's test, and cultural characters, including acid production, of the microbe appeared identical with those of *B. diphtheriae*. Small and large doses of culture caused, on inoculation into rodents, a firm tumor, which gradually enlarged to considerable size, and in the course of a fortnight led to the formation of an abscess. This eventually opened spontaneously and led to complete recovery. No fatal issue in rats or guinea-pigs had been produced by even large doses of the culture. Diphtheria antitoxin, 400 or even 600 units per half-grown guinea-pig, had no power to neutralize the pathogenic action of a small dose of the culture.

The present work was begun in the fall of 1909 at the request of Dr. C. M. Jackson, who had been greatly hampered in certain investigations on white rats because of the death of many of the animals. A routine examination of the dead rats resulted in the constant finding of pneumonitis with consolidation and abscess formation. Some of the lungs did not show distinct abscess formation but only areas of consolidation, resembling lobar pneumonia, with the bronchi filled with a ropy, tenacious mucus and fibrin. The majority, however, were accompanied by abscess formation. These abscesses varied in size from a pin's head to 2 cm. in diameter. They were scattered throughout the lungs and many times occupied an entire lobe. Most of these abscesses were filled with a cheesy pus, although some were filled with a ropy

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[1] *Lancet*, 1903, 1, p. 238; *Centralbl. f. Bakt.*, I, Orig., 1903, 33, p. 488.

mucus like that which was so frequently found in the bronchi. Upon microscopical examination of consolidated areas from rats, dead early in the course of the disease, a bronchopneumonia was found. The bronchi were distended with fibrin, leukocytes, mostly of the polymorphonuclear variety, and desquamated lining cells. The alveoli were filled with the same sort of exudate. Many of these lining cells contained hemosiderin. The capillaries were distended. Sections from the larger consolidated areas of the older cases showed, for the most part, a caseous degenerated material with chromatin detritus scattered through it.

The number of bacteria in smears from all of these lesions varied considerably. In some cases there were few or none, while in others there were numerous bacteria, for the most part in the shape of short granular rods.

Cultures made from the diseased lungs gave an almost constant finding. Blood serum was used for immediate cultures and in nearly all instances the growth, if any, was a pure culture of *B. muris*. The morphology of this organism varied. Fresh cultures showed a short bacillus varying from 1.5$\mu$ to 2.5$\mu$ in length, most of them having an unstained band. Polar granules, or in some cases three to five scattered granules, were present. Others showed two or more unstained bands and, in some cultures, especially in those under cultivation for some time, granular forms predominated. The organism was slightly larger at one end, and the form having one unstained band resembled very closely, at first sight, *Str. pneumoniae*. Some solid forms were nearly always present. In very old cultures branching forms were found. These branches were simple lateral offshoots and various bizarre forms.

## STAINING.

*B. muris* stained well with the ordinary dyes, particularly Loeffler's methylene blue. The granules were well stained by Neisser's method. The organism was gram positive.

## CULTURAL CHARACTERISTICS.

*Broth.*—There was clouding of the media in 24 hours. Upon standing, the bacilli collected at the bottom of the tube. When the tube was shaken they readily diffused through the medium.

## ANIMAL EXPERIMENTS.

| No. of Animal | Method | Kind of Animal Dosage | Date Inoculated | Date Killed or Dead | Pathological Findings | Cultures |
|---|---|---|---|---|---|---|
| 1...... | 1 | Wild mouse, 0.5 c.c. 24-hour broth culture | 6/25/10 | 6/28/10 Found dead | Lungs congested | Negative |
| 2...... | 1 | Wild mouse. Same as No. 1 | 6/25/10 | 6/28/10 | Lungs congested | Negative |
| 3...... | 1 | Guinea-pig. Same as No. 1 | 6/25/10 | 7/21/10 Killed | Negative | Negative |
| 4...... | 1 | White rat. Same as No. 1 | 6/25/10 | 7/1/10 Killed | Negative | Negative |
| 5...... | 1 | White rat, 0.5 c.c. blood serum culture | 7/2/10<br>7/7/10 | 7/25/10 Killed | Negative | Negative |
| 6...... | 2 | White rat, 1 c.c. 24-hour blood serum culture | 6/30/10 | 7/21/10 | Abscess formation at site of inoculation discharging a creamy pus | Positive |
| 7...... | 2 | Common mouse, 0.5 c.c. 24-hour blood serum | 6/30/10 | 7/25/10 Found dead | Lungs consolidated. Two abscesses in the ventricles of the heart. Large abscesses on the diaphragmatic surface of the right lobe of liver, large abscess in the head of the spleen, multiple abscesses in the kidneys | Positive from, lungs, liver, spleen, kidney, and heart |
| 8...... | 2 | Common mouse. Same as No. 7 | 7/16/10 | 7/25/10 Killed | Abscess at the site of inoculation | Positive |
| 9...... | 2 | White rat, few drops of 48-hour broth culture | 7/18/10 | 7/25/10 Killed | Abscess at the site of inoculation measuring 1 cm. in diameter | Positive |
| 10...... | 4 | Guinea-pig 1 c.c. 48-hour broth culture | 7/18/10 | 7/25/10 | Induration at the site of inoculation and abscess formation | Positive |
| 11...... | 3 and 4 | White rat, 0.5 c.c. 48-hour broth culture | 7/19/10 | 7/22/10 Found dead | Yellowish-white purulent collections scattered over the peritoneum. Pin head in size. Granular fibrino-purulent exudate causing adhesion of pleurae of both lungs. Abscess in the wall of the thorax at the site of inoculation. Deep red consolidated areas in both lungs. Fibrino-purulent pericarditis | Positive from peritoneum, pleura, kidney, and lungs |
| 12...... | 3 and 4 | Guinea-pig, 0.5 c.c. 24-hour agar culture | 10/1/10<br>10/12/10 | 11/1/10 Killed | Negative | Negative |
| 13...... | 2 | Guinea-pig. Same as No. 12 | 10/1/10 | 11/1/10 | Abscess following at site of injection which healed spontaneously. Otherwise negative | Negative when killed |

ANIMAL EXPERIMENTS—*Continued.*

| No. of Animal | Method | Kind of Animal Dosage | Date Inoculated | Date Killed or Dead | Pathological Findings | Cultures |
|---|---|---|---|---|---|---|
| 14...... | 3 and 4 | Domestic rat, 0.5 c.c. 24-hour agar culture | 10/1/10 | 10/7/10 Found dead | Small abscess in the abdominal wall. Large abscess in the right thoracic wall. Granular fibrino-purulent exudate causing adhesion of pleurae of both lungs. Lungs congested and pneumonic. Fibrino-purulent pericarditis | Positive from pleura, peritoneum, and heart's blood |
| 15...... | 5 | Guinea-pig | 10/9/10 | 12/5/10 Killed | Negative | Negative |
| 16...... | 4 | Domestic rat, few drops of 24-hour agar culture | 11/7/10<br>11/17/10 | 12/7/10 Killed | Abscess formation few days after first injection discharging creamy pus<br>Discharging fistula connecting with subcutaneous abscess and abscess in right lung measuring 1 cm. in diameter | Positive from site of inoculation and lung abscess |
| 17...... | 4 | Domestic rat, 1 c.c. agar culture | 11/17/10 | 12/2/10 Found dead | Abscess measuring 4 mm. in upper pole of right kidney | Positive |
| 18...... | 3 | Domestic rat, 1 c.c. agar culture | 11/17/10 | 11/24/10 Found dead | Abscess in thoracic wall. Granular fibrino-purulent exudate causing adhesion of the pleurae of both lungs. Pneumonia. Fibrino-purulent pericarditis | Positive from site of inoculation, lungs, and heart's blood |
| 19...... | 3 | White rat, 0.5 c.c. 24-hour agar culture | 1/12/11 | 1/14/11 Found dead | Bilateral granular fibrino-pleuritis. Lungs consolidated. Fibrinous pericarditis | Positive from pleural cavity and heart's blood |
| 20, 21, 22 | 6 | White rats, 1 c.c. filtrate of 10-day lactose broth culture in right pleural cavity | 2/3/11 | 3/10/11 | Negative | |

*Gelatin stab.*—A granular streak along the line of inoculation; no liquefaction.

*Agar streak.*—The organism grew along the line of inoculation as very small white granular colonies with a smooth border and with little tendency to coalesce.

*Blood serum.*—Granular colonies similar to those on agar, but larger. Where widely separated, the colonies became much larger.

*Hiss's serum water.*—Acid reaction in dextrose and maltose, but not in saccharose and lactose.

*Litmus milk.*—No change.

## ANIMAL EXPERIMENTS.

For clearness in the table of experimental data, each method used will be described and given a number.

1. Suspensions of the organism were injected into the mouth and nose of the animal in an attempt to produce lesions in the lungs by inspiration.
2. Subcutaneous injections of bacillary suspensions were made for the study of the local reaction and in an attempt to produce a general infection.
3. Intrapleural injections of bacillary suspensions were made in order to study the reaction of the pleura and lung tissue.
4. Intraperitoneal injections of bacillary suspensions were used to study the effect on the abdominal viscera.
5. Moistening the food with bacillary suspensions, to see if the disease was transmitted in this manner.
6. Injections of the filtrate of lactose broth cultures, to see if a soluble toxin was produced.

## GENERAL DISCUSSION.

With method 1, all of the animals gave negative results. With method 2, however, positive results were obtained in every case. Most of the animals had the same lesion as described by Klein, but the injection into animal 7 resulted in a septicemia and general infection. The results of intrapleural injections established the fact that *B. muris* was fatal to rats when injected at the proper site. Those dying as a result of intrapleural injections showed the same lesions; namely, a granular fibrino-purulent exudate causing adhesion of the visceral to the parietal pleura, consolidated areas in the lungs, and a granular fibrino-purulent pericarditis. The results obtained in animal 16 resemble more closely the natural disease. That the animal did not die was due to the establishment of a permanent fistulous tract between the lung and the outside world. The extent of lesions in animal 17 is rather insignificant to produce a fatal result, yet the presence of a nephritic abscess from which a pure culture of *B. muris* was obtained must be accepted as the cause of death. None of the animals injected with the lactose broth filtrate gave any evidence that a soluble toxin was produced.

Microscopical examination of the so-called "abscesses" which occurred in the kidneys, spleen, and liver, and the granular collections on the pleura, pericardium, and peritoneum showed them to consist mainly of a growth of *B. muris*. These findings are very similar to the "drüsen" formation of actinomycosis and also to the lesions produced by many bacteria belonging to the group of diphtheroid and pseudo-tuberculosis bacilli.

Many diphtheroid bacilli and pseudo-tuberculosis organisms are pathogenic for the lower animals, particularly rodents. They are all very similar to *B. muris*. The bacillus of pseudo-tuberculosis of mice is described by Kutscher[1] as follows: "Morphologically, it resembles the diphtheria bacillus, staining irregularly, appearing in bizarre forms, grows readily, does not form spores, does not liquefy gelatin, is gram positive, and proved pathogenic only for mice." Reed[2] in working with this same organism confirmed the findings of Kutscher. Part of her summary is as follows:

> 1. We may conclude that several different bacteria are concerned in the bacillary pseudo-tuberculosis of animals.
> 2. Rodents seem especially liable to such forms of tuberculosis.
> 3. The bacilli described by Kutscher and Welch are identical.
> 4. The experimental production of the natural disease in mice can be accomplished by injections of pure cultures of the bacilli. The only certain methods are inoculation into the pleural and peritoneal cavities.
> 5. The pseudo-tubercles of the disease differ from those previously described in being composed essentially of colonies of bacteria and to a small extent, only, of proliferated and emigrated body cells. The propriety of the denomination "pseudo-tuberculosis" is therefore open to question.
> 6. The bacilli occur in the form of simple and branching rods; the branching is observed in growths in the animal and those in artificial culture. The bacillus closely resembles, both morphologically and in culture, the Klebs-Loeffler bacillus, but can be distinguished from the latter.

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[1] *Ztschr. f. Hyg.*, 1894, 18, p. 327.

[2] *Contributions to the Science of Medicine by the Pupils of William H. Welch*, Baltimore, 1900.

*B. muris* differs from the above in the following ways: It is stained nicely by Neisser's method, it is pathogenic for the wild mouse, domestic rat, guinea-pig, and the white rat.

Purulent lesions are very common in the lungs of rodents to which, as yet, no specific organism has been assigned. McCoy and Currie[1] describe these lesions in a bulletin issued by the Public Health and Marine Hospital Service. An extract from a letter from Dr. McCoy is as follows: "On several occasions I have inoculated guinea-pigs from purulent lung lesions of rats but always with negative results. I thought it just possible that they might represent the lesions of plague. This, however, was not the case. Purulent lesions of the lungs are very common among rats here. I should say that at least one per cent of those examined in San Francisco are affected in this manner."

*B. muris* was found only in the lungs of rats that were suffering from pneumonia. In many of these animals abscesses were also present, but it could not be said that *B. muris* caused the abscesses. In animals in which no abscesses existed, but only the pneumonic condition, *B. muris* was isolated in pure culture. Since the beginning of this investigation the fatal epidemic among the white rats has disappeared. Many of the remaining animals, killed for other research work, show abscess formation in their lungs but no pneumonia. In none of these has it been possible to isolate *B. muris*.

## CONCLUSIONS.

1. *B. muris* is the etiological agent in a specific kind of pneumonitis in white rats.
2. *B. muris* is pathogenic for the white rat, the domestic gray rat, the common mouse, and the guinea-pig.
3. In the case of the guinea-pig the infection remains local.
4. Instances of a general infection occur, as in animal 7.
5. *B. muris* belongs to the group of diphtheroid or pseudotuberculosis bacilli.

In this investigation I have been greatly aided by suggestions from Dr. C. M. Jackson of the University of Missouri and Dr. Edwin O. Jordan, of the University of Chicago, and I take this opportunity of thanking them.

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[1] *Pub. Health Bull. No. 30*, U.S. Public Health and Marine Hospital Service, Washington, 1910.